Reliability Analysis of Mixing Tree for Automated Sample preparation on Digital Microfluidic Biochips

A Dissertation

Submitted in partial fulfilment of the

requirements for the award of the degree

Of

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in

COMPUTER SCIENCE AND ENGINEERING

Submitted By

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DECLARATION OF AUTHORSHIP

I hereby declare that the work, which is presented in this dissertation report entitled "**Reliability Analysis of Mixing Tree for Automated Sample Preparation on Digital Microfluidic Biochips**" towards the partial fulfilment of the requirements for the award of the degree of Master of Technology with specialization in Computer Science Engineering submitted in the department of Computer Science and Engineering, Indian Institute of Technology, Roorkee (India) , is an authentic record of my own work carried out during the period of August 2015 to May 2016 under the guidance of Dr. Sudip Roy, Assistant Professor, Department of Computer Science and Engineering, Indian Institute of Technology Roorkee.

I have not submitted the matter embodied in this dissertation for the award of any other degree or Diploma.

DATE: ……………….. SIGNED: …………………….

PLACE: ………………. (RUPESH KUMAR)

CERTIFICATE

This is to certify that the statement made by the candidate is correct to the best of my knowledge and belief.

SIGNED: …………………….

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Place: Roorkee Assistant PROFESSOR

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I am also highly indebted to all my friends, and my family who gave me the moral support and valuable suggestions. On a personal note, I owe everything to the almighty.

RUPESH KUMAR

ABSTRACT

Sample Preparation is the most necessary step in biochemical applications. Various biochemical reactants are mixed together to produce mixture with target concentration. Many algorithms have been proposed for reactant minimization and to reduce tranportation time during sample preparation on DMFBs in the recent years. In recent years, it have been seen that there is a fault in mixture hardware on DMFBs. Due to fault on mixture, droplets are not mixed homogeneously during sample preparation on DMFBs. Due to non-homogeneous mixing different part of mixed droplet may contain different concentration of reactants. There are various algorithm developed for sample preparation of biochemical assay but none of them are aiming reliability purpose due to hardware fault. We implemented new technique Monte Carlo Simulation aiming reliability during sample preparation by using different existing algorithm on DMFBs . We are using Monte Carlo Simulation in Min-Mix[1], RMA[8] and MTCS[2] algorithm for sample preparation if there is an inhomogeneous mixing on hardware mixture. It uses mixing tree constructed by different algorithm and propagate error on each mixing node then calculate actual concentration due to error. We observed that MTCS gives better performance than Min-Mix and RMA because it uses common sub-tree, which creates a re-convergent fan out in the tree, due to which it reduces error. Furthermore, Monte Carlo Simulation technique can be implemented to other single target sample preparation as well as multi target sample preparation. So this technique is very useful to know which particular algorithm is more reliable for a specific ratio if there is an inhomogeneous mixing in the hardware mixture on DMFBs.

Contents

Table of Figures

LIST OF TABLES

1. INTRODUCTION

1.1. Background

DIGITAL microfluidic biochip (DMFB) is the technology used to assemble droplet based operations on biochips [1]. It makes use of a two dimensional array, which is microfluidic, apart from that, it uses reservoirs, dispensing ports and detectors. Figure 1(a) explains a microfluidic array, which is a made of group of cells. Which are made of two parallel plates and electrodes. In this method according to the electro-wetting principle on dielectric, an electrode is activated to move a droplet to the neighboring electrode. The process is described in figure 1(b) and 1(c). In some recent experiments DMFB has been used. Some such experiments are immunoassay, protein crystallization and DNA sequencing. Sample preparation, which is a front end operation of this method plays a crucial role in biochemical engineering and laboratories [4,5]. As compared to the conventional laboratories, which needs manual intervention, DMFBs use droplets in nanoliter and picoliter of volume, which provides higher sensitivity and less errors. In biochemical assays and experiments, the automation and efficiency of sample preparation causes impact on efficiency and accuracy of the experiment. For example hundreds of solutions need to be prepared for experiments like DNA sequencing and protein crystallization [6].

1.2. Sample preparation

Sample preparation also plays a crucial role in diagnostics. Various biochemical reagent are mixed together to produce mixture with target concentration. Since cost of reagent play major role in biological assay, their wastage should be reduced whenever possible. In recent years, lab-on-a-chip (LOC) becomes most interesting research topic in biochemical application. An LOC perform various applications in a small chip with many advantage like reactant minimization, portability, fast analysis, accuracy. Various algorithms developed for sample preparation with following advantage. Various mixing algorithm like Min-Mix, RMA, MTCS, CODOS are implemented to minimize the wastage of reactants as well as to minimize transportation and scheduling time.

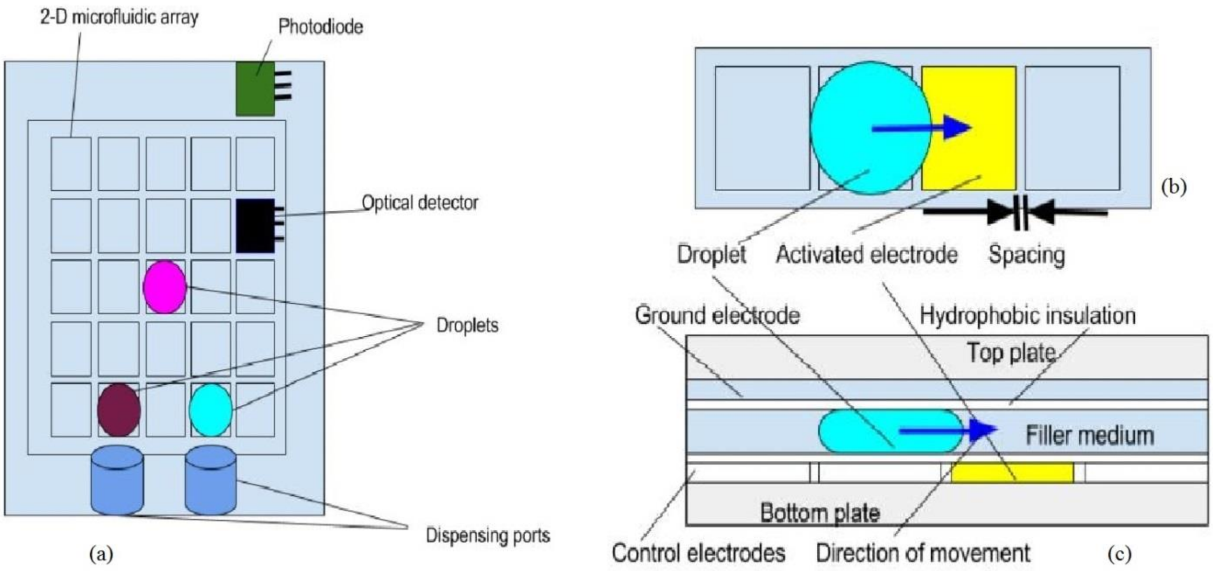

Figure 1 (a) Schematic of a DMFB. (b) Top view of the 2-D microfluidic array. (c) Cross-sectional view of the 2-D microfluidic array [7].

1.3. Problem Statement

Sample preparation is a basic step for all biochemical reactions. Many algorithms have been proposed for reactant minimization and to reduce transportation time during sample preparation on DMFBs in the recent years. In recent years, it have been seen that there is a fault in mixture hardware on DMFBs. Due to fault on mixture, droplets are not mixed homogeneously during sample preparation on DMFBs. Due to non-homogeneous mixing different part of mixed droplet may contain different concentration of reactants. So after splitting both part of mixed droplets may contain different concentration of reactants. There are various algorithm developed for sample preparation of biochemical assay but none of them are aiming reliability purpose. Now main objective is that which algorithm is more reliable whenever fault on the hardware mixture. There are two types fault may occur in the mixture: a) Due to non-homogeneous mixing i.e. different part of mixed droplet may contain different concentration of reactants but equal volume. b) Due to unbalanced splitting i.e. different parts of mixed droplet having same concentration of reactants but unequal volume. In the ideal condition, two unit volume reactants R_1 and R_2 are mixed together, if there is a homogeneous mixing and balanced splitting then the concentration of R_1 and R_2 in the both part of mixture droplet will be (1:1)/2. But if there is non-homogeneous mixing on the mixture with error e=0.1, then the concentration of R1 and R2 in different part of mixed droplet will be (1.1: 0.9)/2 and (0.9: 1.1)/2 . Here both part of droplet having same volume but different concentration of reagents. But if there is a homogeneous mixing and unbalanced splitting then the concentration of R1 and R2 will be (1.1: 1.1)/2 and (0.9: 0.9)/2. Here both part having same concentration but different volume. We implemented new technique Monte Carlo Simulation aiming reliability during sample preparation by using different existing algorithm on DMFBs . We are using Monte Carlo Simulation in Min-Mix, RMA and MTCS algorithm for sample preparation if there is an inhomogeneous mixing on hardware mixture. It uses mixing tree constructed by different algorithm and propagate error on each mixing node then calculate actual concentration due to error. This technique used for reliability analysis of different sample preparation algorithm for any specific ratio if there is an inhomogeneous mixing in the hardware mixture on DMFBs.

2. LITERATURE SURVEY

2.1. Related Work and Prior Paper Contributions

A number of methods have been published recently for sample preparation on DMFBs [1]–[3]. Every algorithm is developed for reagent saving but none of these algorithm is developed for error analysis. Since accuracy and reliability in the sample preparation also plays a major role in various fields.

2.2. Dilution and Mixing Using DMFBs

Interpolation dilution and exponential dilution are the two commonly used dilution methods in laboratory protocols. Interpolation dilution is the key mixing step of sample preparation. One unit volume droplets from each of the concentrations (C1 and C2) is taken to mix and form a mixture of two unit volume. This is done in each interpolation dilution step and then this mixture is spitted again to form two droplets of one unit volume each of concentration (C1+C2)/2. The process is explained in figure 2(a). One unit volume neutral buffer solution is mixed with one unit volume of pure reagent R1of 100% concentration, in each exponential dilution step. In this way after each step we get two unit volumes of half the concentration that we had in previous step. After n steps the concentration of the mixture will remain $1/(2)^n$, if the initial concentration is 100 %. This process is explained in figure 2(b).

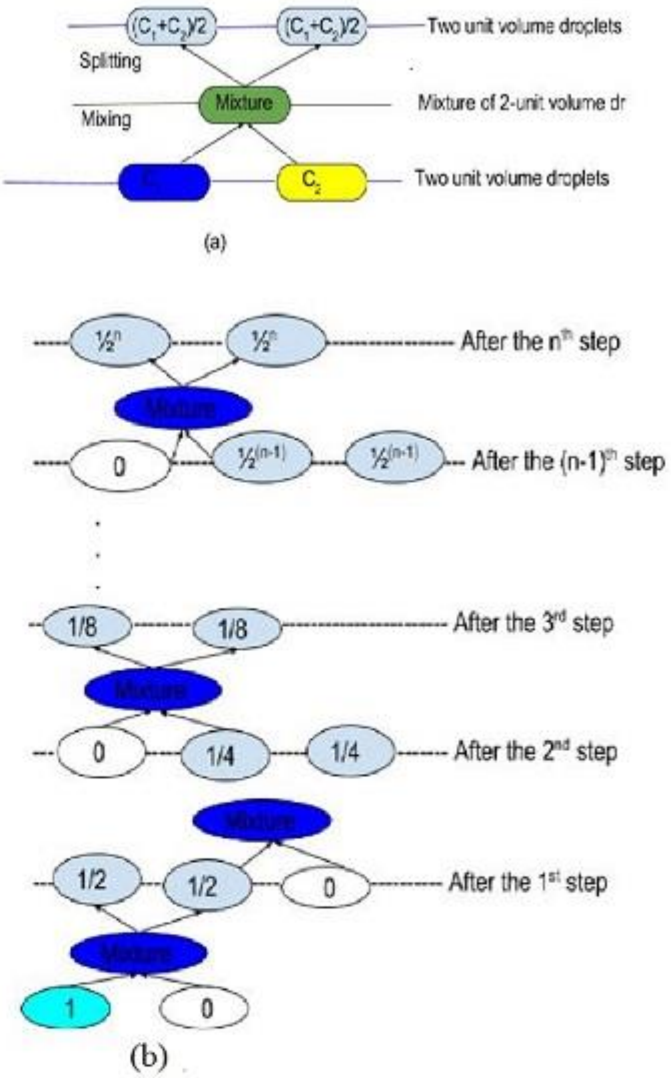

Figure 2 Illustration of two types of dilution methods. (a) Interpolation dilution. (b) Exponential dilution[7]

If two reagents are mixed together then it called dilution but mixing is the procedure if two or more reagents are mixed together. Dilution is the special case of mixing process of sample preparation. We included four mixing algorithms Min-Mix [1], RMA[8], MTCS [2] and CoDOS[3] here . Min-Mix [1] uses d-bit binary fractions for a concentration factors to construct mixing tree of height d after level- wise pairing of nodes. Suppose, we have N reagents to mixed with target concentration a_1:a_2:a_3:.....:a_N where L(Ratio Sum)=2^d and d is the precision level. In this method, It creates d-bits binary fraction for all N ratios due to this it uses N×d bit matrix. It uses bit scaning method from right to left column wise and any column represents the node of same level. Suppose, two pair nodes of level K are mixed together and the resultant mixture node will be the level of (K+1). For example target ratio 2:3:5:7:11:13:87 of seven reactants are R_1, R_2, R_3, R_4, R_5, R_6, and R_7. The seven bit binary fractions are 0.0000010_2, 0.0000011_2, $0.0000101_{2,}$ $0.0000111_{2,}$ $0.0001011_{2,}$ $0.0001101_{2,}$ and 0.1010111_2 respectively. Figure 3 shows mixing tree determined by Min-Mix[1].

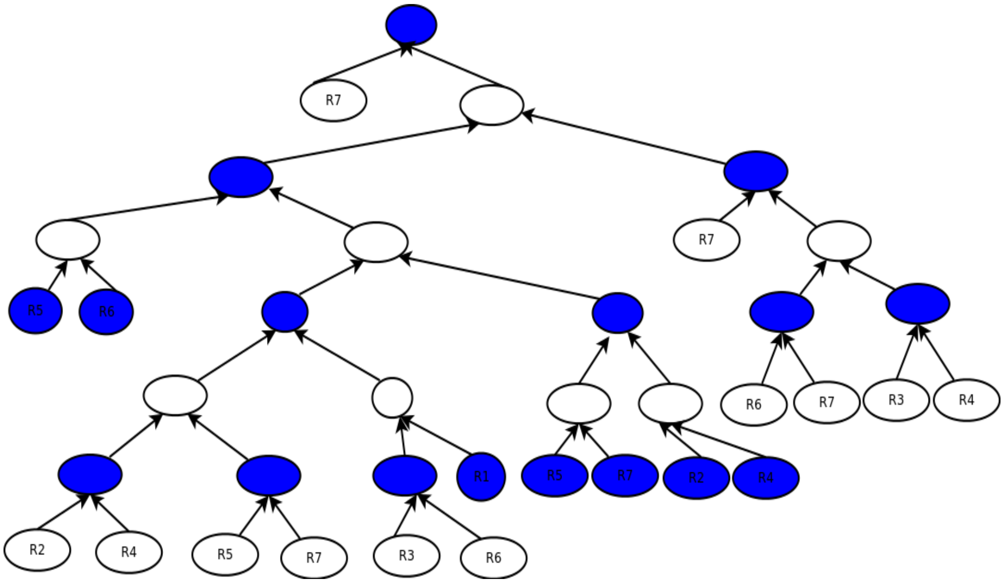

Figure 3 Min-Mix Mixing Tree for target concentration 2:3:5:7:11:13:87

Another mixing algorithm Ratioed Mixing Algorithm RMA[8], this algorithm is implemented for the purpose to minimize transportation cost. The reservoirs are used for supplying different reactants and wash fluids or waste collector are located at the boundary of a biochip. As shown in Fig. 5, seven reservoirs R_1,......,R_7 are located at the peripheral of the electrodes array for the purpose of loading the reagents and two waste collector reservoirs for collecting mixed waste fluids from the chip during sample preparation. The arrow indicates some possible fluid transportation ways from and to the reservoirs. Suppose there are a reservoir for each type of fluid in the chip. Then, for the purpose of mapping mixing tree into a biochip, there are two tasks to be performed: (i) one to one mapping of different type of components to the reservoirs. (ii) Many to one mapping of nonleaf nodes to the chip mixers. If the number of existing mixture modules is less than the number of intermediate mixing droplets then storage units are required on chip for storing intermediate droplets. In Fig. 5, there are cross-contaminated marked by small dots. Three electrodes are cross-contaminated during execution of the five mixing steps in the subtree of the given tree. For this mixing tree, the mixer assignment shown in Fig. 4 and the reservoir allocation shown in Fig. 5(b) can be obtained using a greedy method. By using greedy method, the number of cross-contaminated electrodes decreases due to this washing time also reduces. Mixture assignment and reservoir allocation depends upon the characteristics of mixing tree. If two set having less number of intersection of reagents then it will be favorable for a specific reactants to be mixed. This algorithm provides a mixing tree having most suited placement and reservoir allocation on the biochip. Hence in this algorithm, the expression of a target concentration can be decomposed in terms of subtree having same set of reagents and two subtrees having less number of intersection of reactants. Each fractional decomposition of the expression for a target ratio can be represented by a mixing tree.

Another mixing algorithm, MTCS [2] determines a common sub-tree as the same sub-tree with same labels at the leaf-nodes having two occurrences in a mixing tree. Figure 6 shows mixing tree produced by MTCS. It may be noted that, if there is a common sub-tree in the mixing tree, then the extra intermediate droplet (denoted by the root of that sub-tree) produced at a lower level can be used at a higher level instead of producing the same intermediate droplet again by performing the same set of mix-split steps. It explains the reuse of a waste droplet corresponding to the root of a common sub-tree at a lower level as the leaf node at a higher level. To achieve this concentration MTCS [2] is more efficient than Min-Mix [1] because Min-Mix waste 17 droplets but MTCS waste only 11 droplets. All intermediate nodes indicate waste droplet in Min-Mix but all unshared intermediate node in MTCS indicate waste droplet.

Another mixing algorithm, CoDOS [3] is also similar to MTCS [2]. It also determines a common sub-tree to detect a rectangle in from a recipe matrix. An N×d recipe matrix M specifies a target concentration T where N is a number of reactants type and d is the precision level. For example the mixing tree for target ratio 2: 3: 5: 7: 11: 13: 87 using this method is shown in Figure 7.

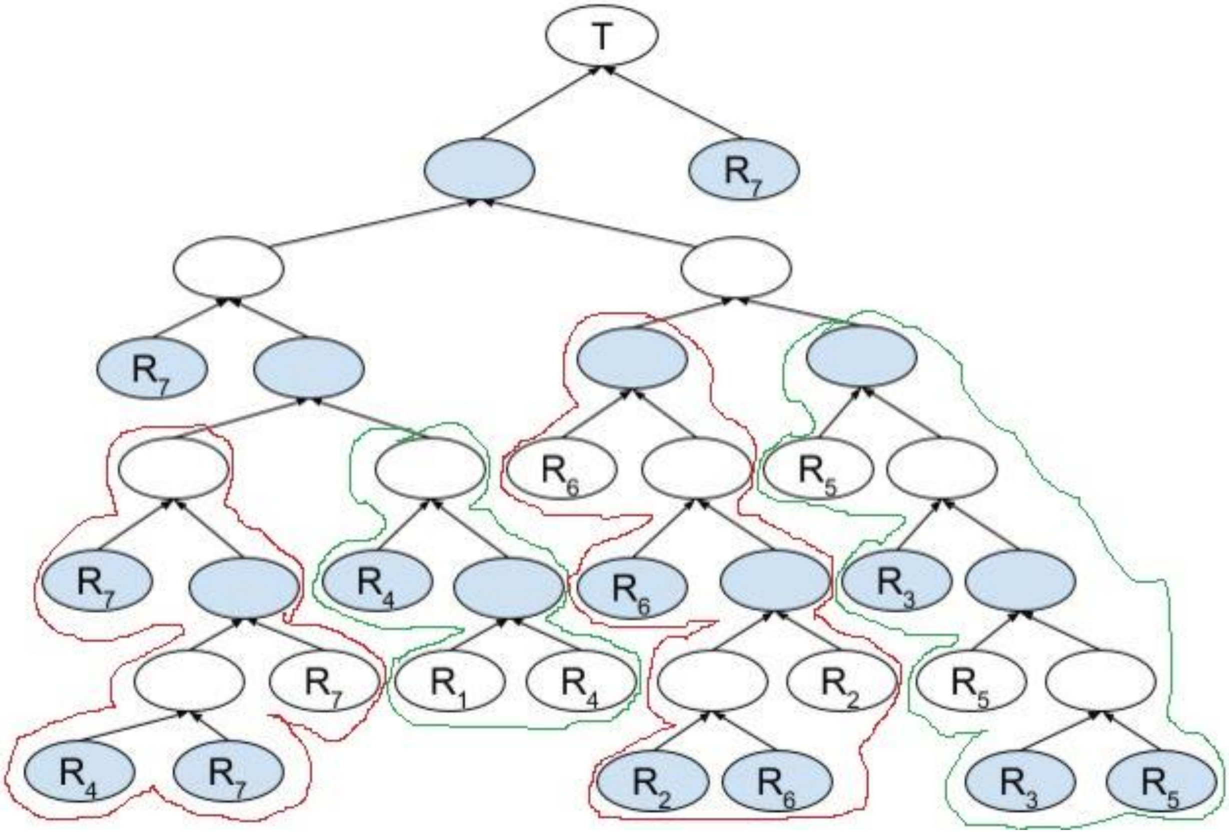

Figure 4 Mixing tree for the example ratio 2 : 3 : 5 : 7 : 11 : 13 : 87 obtained by algorithm RMA[8].

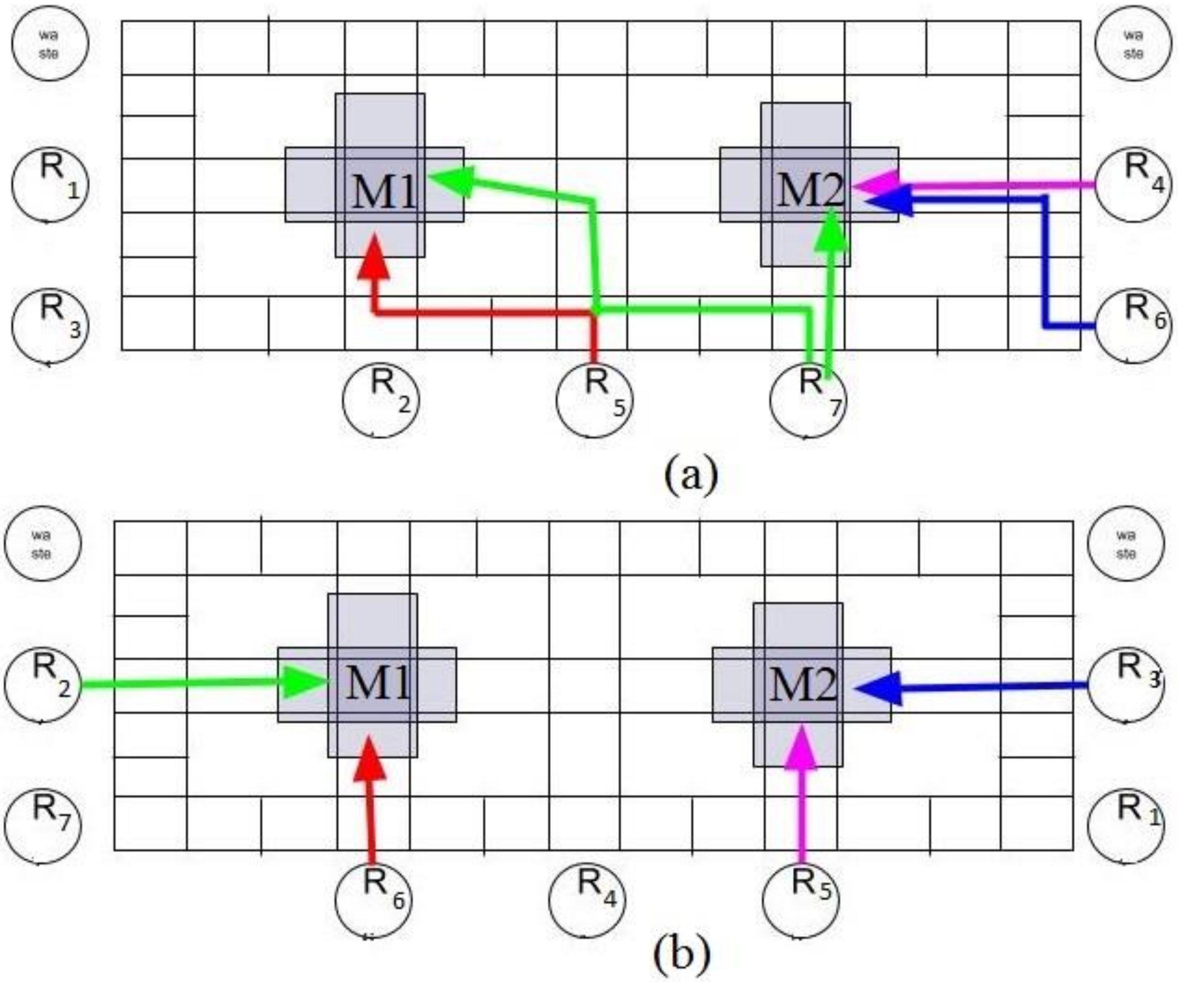

Figure 5 Droplets routes for the mixing steps of the subtree rooted at t13 and t14 in RMA[8].

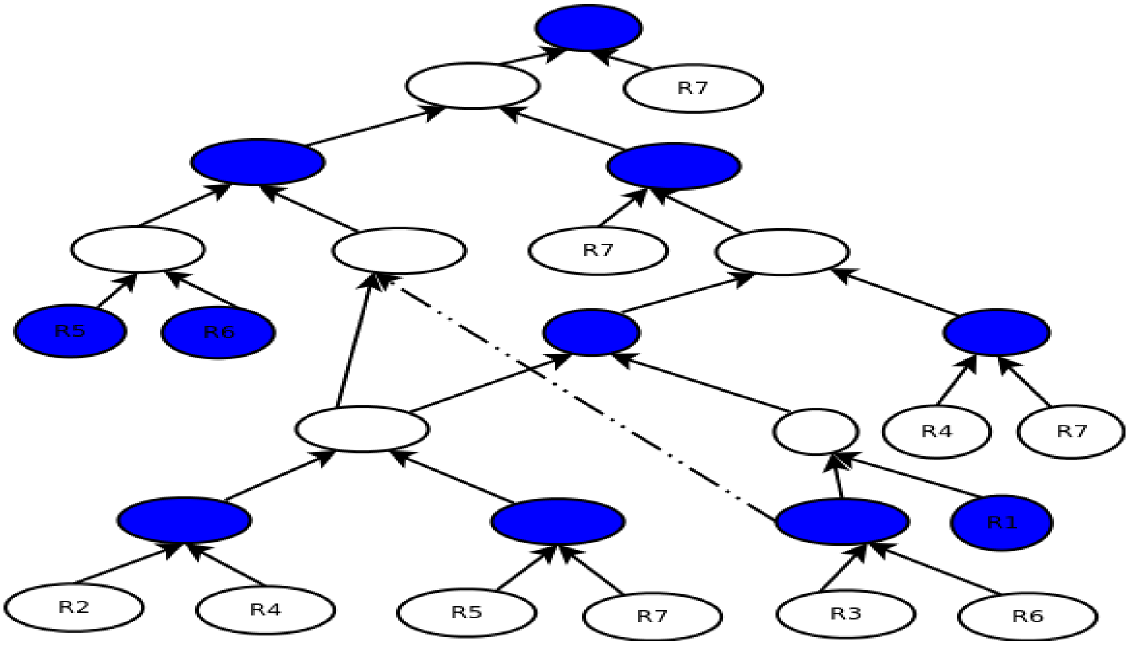

Figure 6 Mixing tree for the example ratio 2 : 3 : 5 : 7 : 11 : 13 : 87 obtained by algorithm MTCS[2].

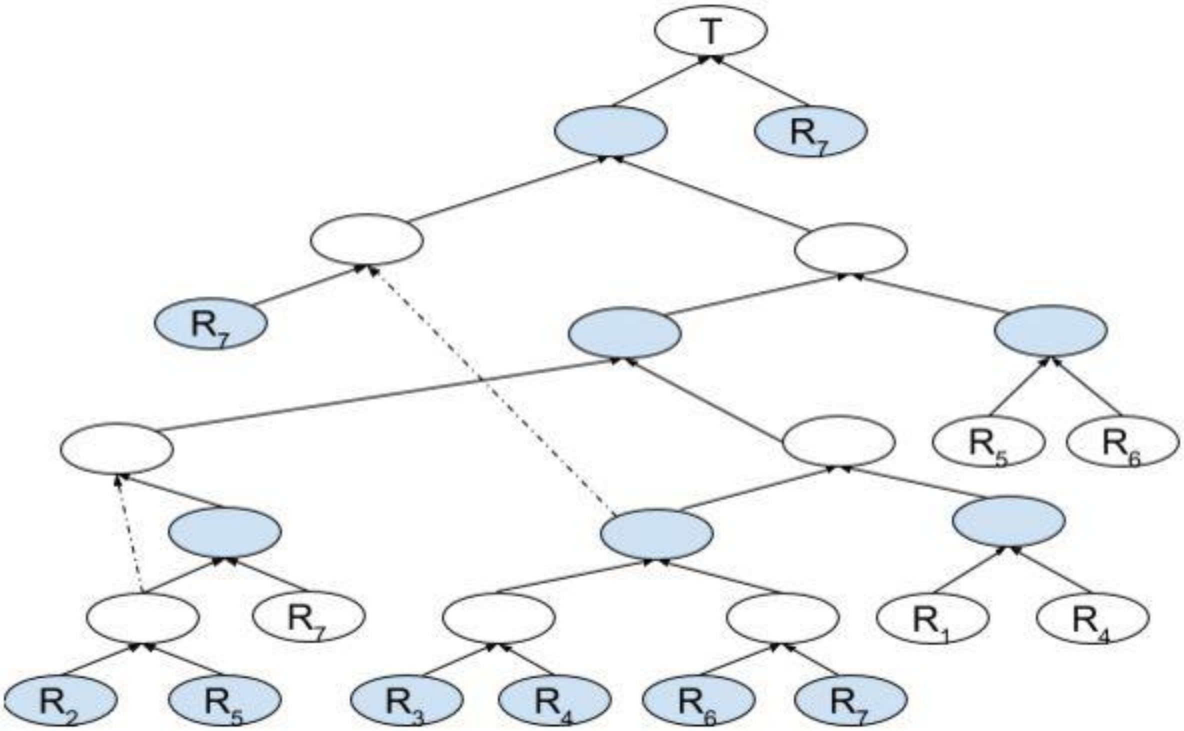

Figure 7 Mixing tree for the example ratio 2 : 3 : 5 : 7 : 11 : 13 : 87 obtained by algorithm CoDOS[3]

3. ANALYTICAL MODEL FOR ERROR ANALYSIS

Analytical model is a mathematical modeling technique. We are doing error analysis on sample preparation due to fault in the hardware mixture and we know that error in the real scenario comes in normally distributed manner. First of all we have to construct a mixing tree using given algorithm then we are taking error in terms of $E_i=N(0, \sigma_i^2)$, where 'i' iterates number of mixture nodes. Mean of error in the node is 0 and standard deviation is σ_i for ith node. Suppose, four reagents are mixed together with target concentration 3: 3: 4: 6 shown in Figure 8. Two reagent R_1 (r_1, 0) and R_2(r_2, 0) mixed together at mixture node $M_{(1,2)}$ with error $E_1= N(0,\sigma_1^2)$ then, mean and standard deviation of node $M_{(1,2)}$ will be as follows.

$$M_{(1,2)}=r_1+r_2+E1=N(r_1+r_2, \sigma_1^2)$$

Now after splitting it becomes two node M_1, M_2.

$$M_1= M_2 = M_{(1,2)}/2=N((r_1+r_2)/2, \sigma_1^2/4)$$

$$M_{((1,2),4)} =R_4+M_1+E_2= N(r_4+M_2, \sigma_1^2/4 +\sigma_2^2)$$

$$M3= M_{((1,2),4)} /2= N(r_1+r_2+2r_4/4, \sigma_1^2/16 + \sigma_2^2/4)$$

$$\text{Similarly } T= N(3r_1+3r_2+4r_3 +6r_4/16, \sigma_1^2/256 + \sigma_2^2/64+ \sigma_3^2/16+ \sigma_4^2/4)$$

Suppose, there are n reagent mixed together with target concentration a_1: a_2: a_3:......: a_n having n bit precision and construct mixing tree with m mixture nodes using given algorithm. Now we are taking error in terms of $E_i=N(0, \sigma_i^2)$ for all mixture nodes from 1 -m. Then standard deviation of error will be in the function of $F(\sigma_1^2, \sigma_2^2,\sigma_3^2 ,..., \sigma_m^2)$.

$$F(\sigma_1^2, \sigma_2^2,\sigma_3^2 ,..., \sigma_m^2)=k_1* \sigma_1^2 +k_2*\sigma_2^2+k_3*\sigma_3^2 +.....+k_m*\sigma_m^2 .$$

Where k_1, k_2, k_3,...., k_m are constant and it is calculated by the above probabilistic method.

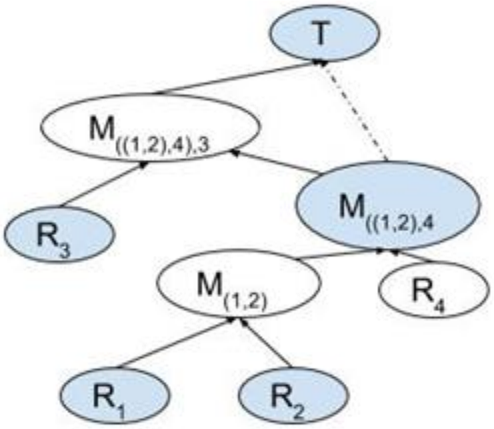

Figure 8 Mixing Tree of sample preparation for the ratio 3:3:4:6

4. MONTE CARLO SIMULATION

Monte Carlo simulation is a mathematical model which allows us to register for risk in mathematical analysis and decision making. Risk analysis is a decision making with ambiguity, uncertainty and variability. In this technique we see all the possible outcomes and determine the impact of risk. This technique is used in various professional field like research and development, engineering, insurance, energy, transportation, project management and the environment. It makes the decision with the range of possible consequences. It uses risk analysis by building model of possible outcomes. It calculates result each time using a different set of random variable from random probability distribution function. Probability distribution is a more realistic way for explaining uncertainties in variables. It is a practical model error analysis so that can also be used in mixing tree for sample preparation. We are using monte carlo simulation here to analyzing error in the target concentration and to determine which algorithm is more reliable if there is fault in mixture hardware. In the sample preparation, different algorithm used to determine target concentration of reagents. Algorithms use different step to get target concentration and different algorithms may use different steps. These stepwise flow constitute mixing tree for target concentration. In the mixing tree, all leaf node represent pure reagents, nonleaf nodes represent mixture node and root node represents target mixture node. So we know that error may occur in the mixture node during mixing of two droplets. It may be possible that there is an fault in hardware mixture. Due to that fault it may occur non-homogeneous mixing during mixing of two droplets in mixture. In the non-homogeneous mixing two different part of mixture droplet contains different concentration of reagents. In the mixing node, all nonleaf node represent mixture node so we have to propagate error all nonleaf nodes for analysing error in the target ratio. We know that monte carlo simulation is a realistic model so we have to propagate error in the realistic manner. We are using random probability distribution to distribute error in the mixing node. Error distribution in the mixing node is taken by random number generator using normal distribution where mean of the error is 0 and standard deviation 0.055. We are taking input as N,T and M where N =Numbers of reagent, T=Target concentration and M= Method(Algorithm) type. Hardware fault indicates, there is a non-homogeneous mixing in the mixture hence due to nonhomogeneous mixing different part of droplet may contain different concentration of reagents. After splitting it becomes two droplet with different concentration of reagents then we keep one part of droplet for next step of mixing and second part of droplet may be waste or use in future as algorithms requirements. In the Min-Mix algorithm second part of mixed droplet is always waste but in the MTCS or CoDOS second part of mixed droplet may be used as algorithms requirement.

we are using a random number generator using normal distribution for distributing error on the mixing nodes. We are taking input as a mixing tree produced by different algorithm (Min-Mix, MTCS and RMA) and applying Monte Carlo Simulation at each mixing tree. Error indicates that there is a non-homogeneous mixing at the hardware mixture and after splitting different part of mixture droplet contains different concentration of reagents. Random error using random probability distribution generates maximum 13% error at any mixture node. Suppose, there are two reagents R_A and R_B mixed together with error 0.1 at mixture node. In homogeneous mixing and balanced splitting, the concentration of R_A and R_B should be (1: 1)/2 in both mixture droplets. But due to non-homogeneous mixing and balanced splitting, the concentration of R_A and R_B will be (1.1: 0.9)/2 and (0.9: 1.1)/2 respectively. In the Monte Carlo Simulation, we taking error E with mean μ=0 and standard deviation σ_i =0.055 for i_{th} node of the mixing tree. Suppose, there is sample preparation having n reagent with target concentration a_1:a_2:a_3…..:a_n with n-bit precision but due to errors on the hardware mixture ,we got mixture ratio with concentration a_1+e_1, a_2+e_2,a_3+e_3,…., a_n+e_n , where 0.13>=e_i>=-0.13 and Σ $a_i=2^n$ for i=1,2,3…,n. After getting mixture with error we calculate Root Mean Square(RMS) value of errors and stored it in the file. RMS of error value is described as $a_{rms}=\sqrt{\frac{1}{n}\sum xi^2}$ where $x_i=(e_i)/2^n$ and RMS mean value is described as $a_{rmsmean}=\sqrt{\frac{1}{n}\sum xi^2}$ where $x_i=(a_i)/2^n$. It is repeated 100,000 times and stored in a file, then we plot graph between all RMS value of errors and graphs have been ploted in normal distribution that also shows our experiment is more realistic. Now we calculate mean and standard deviation of $a_{rms.}$. After caWe applied this techniques on all ratio of ratio sum 16 and 32 respectively. Ratio sum 16, 32 contains 198, 6058 ratios. Better algorithm gives less standard deviation. Some algorithm uses sharing intermediate node that makes re-convergent fan-out. Due to re-convergent fan-out, the error is minimized because both complement ratios are mixed together at some point. That algorithm will be more reliable which is having less standard deviation of errors. We are implementing monte carlo simulation in MinMix, RMA, MTCS. There can be occur different types of other hardware fault like homogeneous mixing of droplets on the mixture but unbalanced splitting. In this problem, the concentration of reagents must be same in the both part of mixed droplets but volume of both part may be differnt. We are also implemented Monte Carlo Simulation during sample preparation on DMFBs due to that problem. After simulation result, we observe that volume of target ratio droplet may not be V unit droplet volume, it may be more or less volume. But in the previous hardware probem volume of target droplet is always V unit volume because there is a balanced splitting. Following flowchart explained all stepwise process of Monte Carlo Simulation.

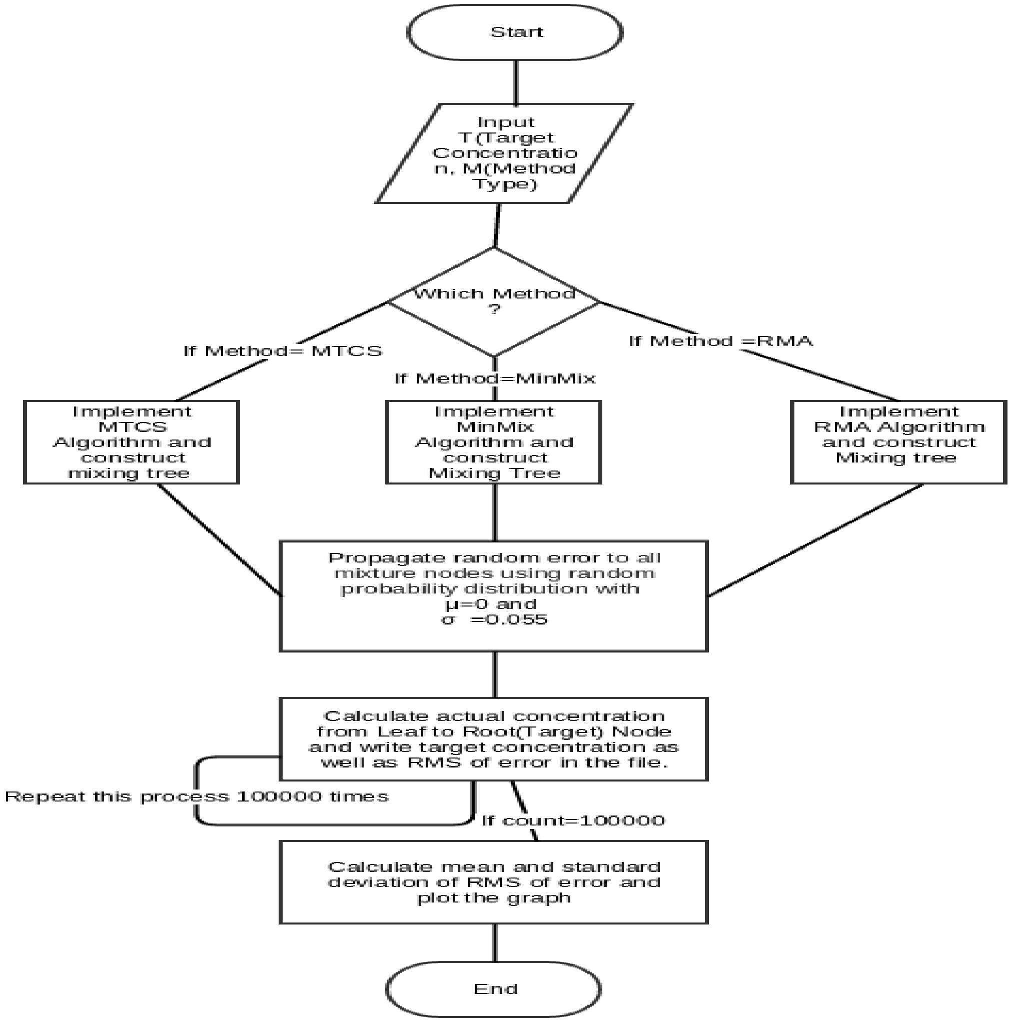
Start
Input
T(Target
Concentratio
n, M(Method
Type)
Which Method
?
If Method= MTCS
If Method=MinMix
If Method =RMA
Implement
MTCS
Algorithm and
construct
mixing tree
Implement
MinMix
Algorithm and
construct
Mixing Tree
Implement
RMA Algorithm
and construct
Mixing tree
Propagate random error to all
mixture nodes using random
probability distribution with
μ=0 and
σ =0.055
Calculate actual concentration
from Leaf to Root(Target) Node
and write target concentration as
well as RMS of error in the file.
Repeat this process 100000 times
If count=100000
Calculate mean and standard
deviation of RMS of error and
plot the graph
End

Figure 9 Flow Chart for Monte Carlo Simulation

5. EXPERIMENTS AND RESULTS

We are implemented Monte Carlo Simulation in Min-Mix, RMA and MTCS algorithm for Ratio Sum (L=8, 16, 32, 64, 128).

Method: Min-Mix, RMA,MTCS (Nonhomogeneous mixing for L=8)

Method	R1(μ, σ)	R2(μ, σ)	R3(μ, σ)	RMS(μ, σ)
Min-Mix	2.000310, 0.155917	2.999700, 0.145232	2.999989, 0.145640	0.016498, 0.008609
MTCS	2.000318, 0.154978	2.999924, 0.095126	2.999758, 0.095299	0.012666, 0.007735
RMA	2.000298, 0.150438	2.999612, 0.189434	2.999452, 0.180056	0.017611, 0.010070

Table 1 Mean and standard deviation calculated for each reactant as well as RMS of error after simulation in Min-Mix ,RMA, MTCS Algorithm (L=8, ratio 2:3:3).

Method: Min-Mix, RMA, MTCS (Nonhomogeneous mixing for L=16)

Method	R1(μ, σ)	R2(μ, σ)	R3(μ, σ)	R4(μ, σ)	R5(μ, σ)	RMS(μ, σ)
Min-Mix	2.999845, 0.190788	3.000131, 0.190328	2.999657, 0.190297	5.000248, 0.330593	2.000119, 0.190685	0.012959, 0.005417
MTCS	3.000943, 0.219331	2.999071, 0.155767	2.999376, 0.155568	5.001135, 0.290879	1.999474, 0.190555	0.011936, 0.005225
RMA	2.999943, 0.211674	2.999874, 0.198374	3.000468, 0.223892	4.999463, 0.339053	2.000437, 0.171176	0.014404, 0.006159

Table 2 Mean and standard deviation calculated for each reactant as well as RMS after simulation in Min-Mix , RMA, MTCS Algorithm (L=16, ratio 3:3:3:5:2).

Method: Min-Mix, MTCS, RMA (Nonhomogeneous mixing for L=32)

Method	R1(μ,σ)	R2(μ,σ)	R3(μ,σ)	R4(μ,σ)	R5(μ,σ)	R6(μ,σ)	R7(μ,σ)	RMS(μ,σ)
Min-Mix	6.99888 0.40122	7.00154 0.36842	5.00042 0.39625	4.99839 0.36901	2.99974 0.25269	3.00090 0.25208	2.00014 0.22060	0.009681 0.003247
MTCS	6.99724 0.38433	7.00212 0.38523	5.00073 0.33501	5.00034 0.33321	2.99970 0.22705	3.00063 0.22622	1.99914 0.21998	0.009132 0.003210
RMA	7.00013 0.447593	6.99695 0.431486	4.99983 0.453219	5.00015 0.383743	3.00067 0.270935	2.99970 0.264679	1.99987 0.248977	0.012081 0.004547

Table 3 Mean and standard deviation calculated for each reactant as well as RMS after simulation in Min-Mix , MTCS, RMA Algorithm (L=32, ratio 7:7:5:5:3:3:2).

Method: Min-Mix, MTCS, RMA (Nonhomogeneous mixing for L=64)

Method	R1(μ, σ)	R2(μ, σ)	R3(μ, σ)	R4(μ, σ)	R5(μ, σ)	R6(μ, σ)	RMS(μ,σ)
Min-Mix	5.000790, 0.433958	7.001002, 0.485518	8.999648, 0.742487	11.001299, 0.776947	12.998122, 0.758489	18.999138, 1.196428	0.011189, 0.004412
MTCS	4.999975, 0.487374	6.999335, 0.460596	9.001448, 0.742621	11.002478, 0.718102	12.995661, 0.943680	19.001103, 1.179852	0.011681, 0.004377
RMA	4.999893, 0.511142	7.000154, 0.499877	9.005233, 0.760013	10.998462, 0.769822	13.002448, 0.961182	18.998361, 1.200085	0.013162, 0.005268

Table 4 Mean and standard deviation calculated for each reactant as well as RMS after simulation in Min-Mix , MTCS, RMA Algorithm (L=64, ratio 5:7:9:11:13:19).

Method: Min-Mix, MTCS, RMA (Nonhomogeneous mixing for L=128)

Method	R1(μ, σ)	R2(μ, σ)	R3(μ, σ)	R4(μ, σ)	R5(μ, σ)	R6(μ, σ)	R7(μ, σ)	RMS(μ,σ)
Min-Mix	1.999086, 0.268791	2.998827, 0.344249	5.000918, 0.514278	6.998618, 0.601059	10.996159, 1.018521	12.996135, 1.045418	87.010256, 2.565068	0.007884, 0.004550
MTCS	1.999987, 0.268479	2.999141, 0.305800	5.002882, 0.488082	7.002781, 0.617986	10.997412, 0.982954	12.999752, 1.099092	86.998046, 2.478078	0.007800, 0.004520
RMA	2.000188, 0.270097	3.000683, 0.355290	4.998832, 0.500097	7.003947, 0.622191	10.979866, 1.022776	13.004892, 1.110983	86.998001, 2.572249	0.008623, 0.004708

Table 5 Mean and standard deviation calculated for each reactant as well as RMS after simulation in Min-Mix, MTCS, RMA Algorithm (L=128,ratio 2:3:5:7:11:13:87).

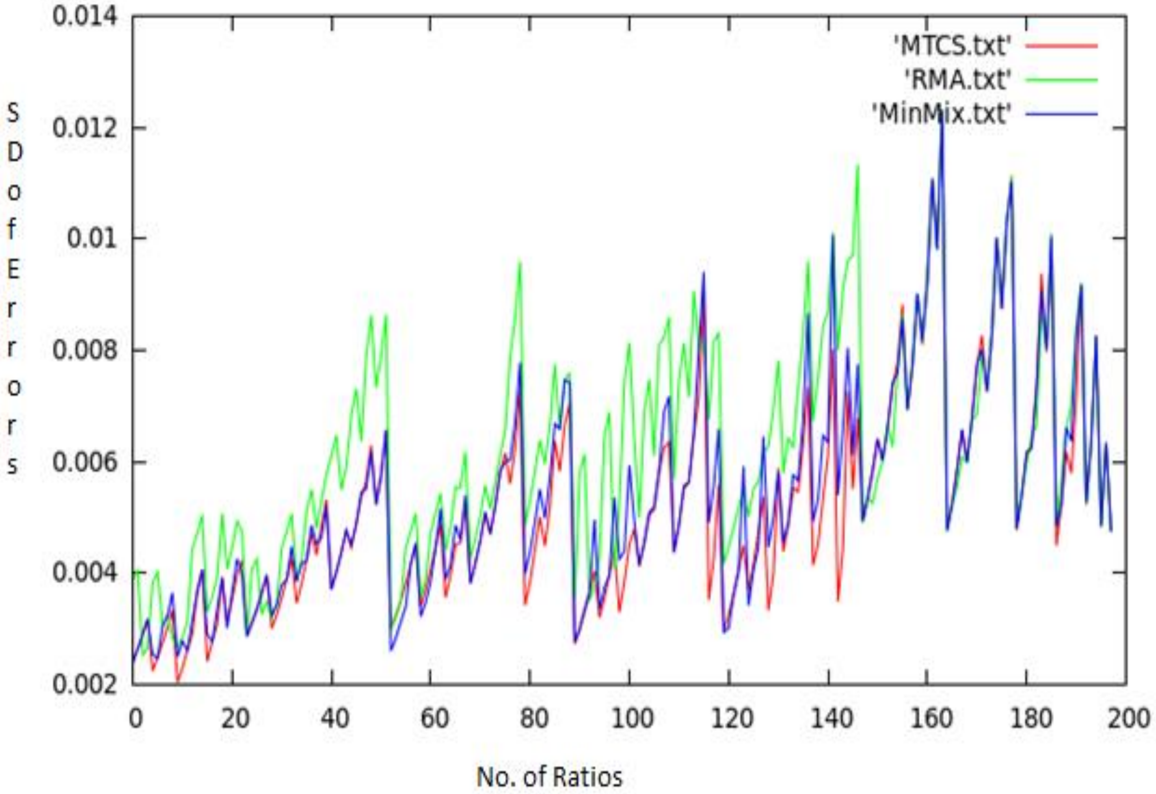

Figure 10 Standard deviation plotted for all 198 ratio of ratio sum 16 with these 3 method MinMix[1], MTCS[2], RMA[8].

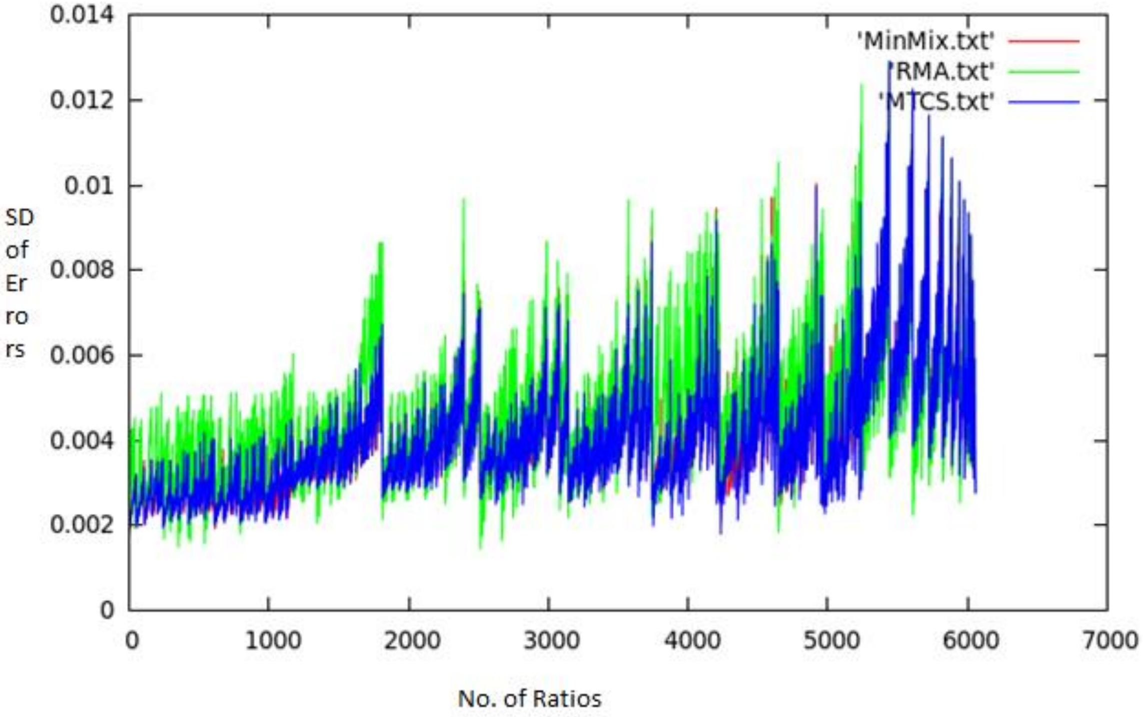

Figure 11 Standard deviation plotted for all 6058 ratio of ratio sum 32 with these 3 method MinMix[1], MTCS[2], RMA[8].

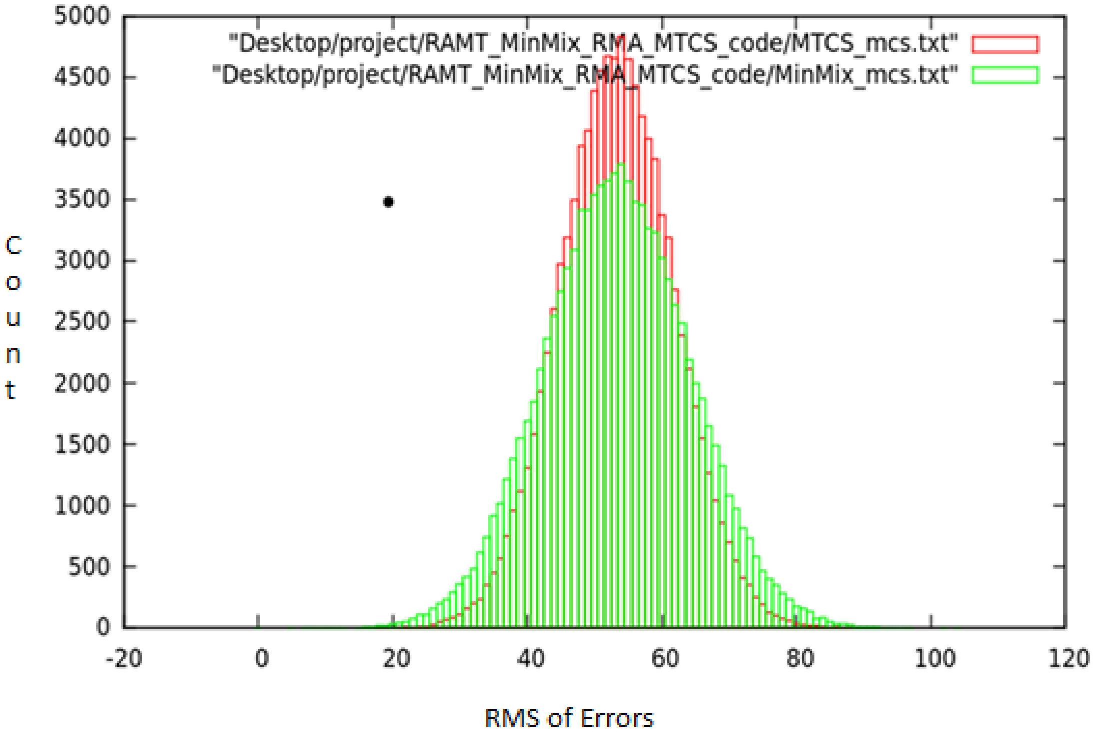

Figure 12 RMS value plotted for Min-Mix (green) and MTCS (red) after Monte Carlo Simulation

6. CONCLUSION

Sample preparation is a basic step for all biochemical reactions. Many algorithms have been proposed for reactant minimization during sample preparation on DMFBs in the recent years. Nevertheless, some of them can control the cost and reactant minimization problem but none of them thinking about reliability problem due to hardware fault. We implemented new technique Monte Carlo Simulation aiming reliability during sample preparation by using different existing algorithm on DMFBs. We are using Monte Carlo Simulation in Min-Mix[1], RMA[8] and MTCS[2] algorithm for sample preparation if there is an inhomogeneous mixing on hardware mixture. It uses mixing tree constructed by different algorithm and propagate error on each mixing node then calculate actual concentration due to error. We observed that MTCS gives better performance than Min-Mix and RMA because it uses common sub-tree, which creates a re-convergent fan out in the tree, due to which it reduces error. So we can say that MTCS is more reliable than Min-Mix and RMA. Furthermore, Monte Carlo Simulation technique can be implemented to other single target sample preparation as well as multi target sample preparation. So this technique is very useful to know which particular algorithm is more reliable for a specific ratio if there is an inhomogeneous mixing in the hardware mixture on DMFBs.

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